

Eastern Equine Encephalitis Surveillance



by Mary Tobin

Since 1938, 74 human cases and hundreds of equine cases of eastern equine encephalitis (EEE) have occurred in Massachusetts. Although rare, human EEE is considered to be the most devastating arthropod-borne encephalitis in North America, as infection often results in severe illness, permanent impairment¹ or death. The causative agent of the disease is eastern equine encephalitis virus (EEEV), an *Alphavirus*, which is transmitted within its avian reservoir by the mosquito, *Culiseta melanura*.

Outbreaks of EEE have occurred in essentially all states east of the Mississippi River and in Minnesota, South Dakota and Texas. In most years in Massachusetts, the virus is found only in its fresh water swamp foci. The convergence of several factors that promote EEEV buildup can result in a high prevalence of active infection in birds and an increased risk of spillover to non-reservoir species. The major variables that affect the level of EEEV are factors associated with mosquito population levels, such as seasonal and annual rainfall and temperature; factors associated with reservoir susceptibility, such as avian immunity and abundance; and factors that affect the chance of transmission to humans, such as emergence of human-biting mosquito species when EEEV is at high levels in its avian reservoir. This confluence of events can cause a chance occurrence in which a bridge vector mosquito feeds on an infected bird and then on a human. Bridge vector mosquitoes transmit the virus from the endemic host birds to the accidental hosts: humans, horses, and other animals.

In response to an outbreak in 1955-56, in which 16 human cases occurred in Massachusetts, the U.S. Centers for Disease Control and Prevention established the EEE Surveillance Program (EEESP) to assess the seasonal prevalence of virus in mosquitoes. During the following 13 years, there were no human cases of EEE in Massachusetts, and the surveillance program transitioned from a federal research project to a state-funded prevention program. The program now has three components: surveillance, risk assessment and risk management. In 1973, the EEESP provided information that gave an early warning of an outbreak, and many cases of EEE were prevented by timely vector control activities. Since 1973, the EEESP has guided policy decisions for vector control and public education to limit the occurrence of EEE.

During the mosquito season, from May through early October, field workers trap mosquitoes throughout the EEE High Risk Area². The traps collect flying insects that are brought back to SLI and the mosquitoes are sorted by species. Female mosquitoes from the same trap are processed in pools of up to 50 each, and the pools are tested for EEE and Highlands J (HJ) viruses.

In addition to surveillance for EEEV, other risk factors are monitored, such as mosquito population, rainfall and groundwater levels. SLI tests suspect human and animal cases of EEEV infection throughout the season. Surveillance data are shared through a CDC sponsored listserver that reports information on arboviral activity throughout the U.S. State public health surveillance programs report information on eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, LaCrosse encephalitis and other arboviruses that cause human illness.

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Polychlorinated Biphenyl Exposure Assessments

by Julianne Nassif

Polychlorinated biphenyls (PCBs) are a class of organic compounds that were widely used in a variety of industrial applications until their ban by the Environmental Protection Agency in 1978. Analysis of PCBs is difficult because there are 209 possible PCB congeners. Quantitative analysis of the individual species of PCBs is important because the congeners have varying physical and chemical properties and associated toxicity. Quantitative analysis is done also without measuring specific congeners by comparison to known mixtures of PCBs or Aroclors. Initial testing often uses the latter procedure for cost and time efficiency. Once a contamination problem has been identified in screening tests, specific congener analysis may be done to better define the exposure source and the health risks.

PCBs were used in the manufacture of electrical products in Pittsfield, a city in western Massachusetts, from 1932 to 1972.

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Public education is an integral part of the surveillance program's risk management effort. In the spring, information on EEEv and ways to prevent mosquito bites is sent to boards of health, camps and campgrounds in the EEE High Risk Area. The education effort is ongoing and intensifies when surveillance data indicate an increased risk of EEEv transmission to humans. As EEEv is identified,

boards of health in and around the area, the media, mosquito control agencies and the State Reclamation and Mosquito Control Board are notified. Strategies and procedures that are described in the "Vector Control Plan to Prevent Eastern Equine Encephalitis", MA DPH, 1991, guide decision-making. Larvaciding of mosquitoes is done in preference to adult-ciding, which requires application of chemical pesticides. Aerial spraying of mosquitoes is an intervention that is not used unless there is a clear indication that the risk of EEE disease is high and that multiple cases would occur unless action is taken. The EEESP has devel-

oped an efficient early warning system that assesses risk on a weekly basis. Weekly reports and seasonal summary reports may be found at the following Web site: www.state.ma.us/dph/bls. Questions and comments can be sent to mary.tobin@state.ma.us.

¹ The economic cost of a single severe case of EEE has been estimated at over \$2 million.

² Plymouth, Norfolk, Bristol, Suffolk and eastern Middlesex Counties

Polychlorinated Biphenyl Exposure Assessments

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PCBs reached the Housatonic River via direct release and indirect discharge through the ground water. PCB contaminated sediments and fish were detected, which led to the issuance of a freshwater fish consumption advisory, warning individuals against eating fish and amphibians from the Housatonic River. Subsequent testing of other environmental media revealed PCB contamination in soil, aquatic plants and ambient air.

The Massachusetts Department of Public Health became concerned about PCB levels for residents living in the eight-town region that comprised the floodplain, given the multiple sources for environmental exposure. Many residents of the area were previously employed by the electrical manufacturer and had opportunity for occupational exposure. As a result, the Bureau of Environmental Health Assessment initiated the Housatonic River Exposure Assessment, and testing was provided at the Environmental Chemistry Laboratory of SLI. The Federal Agency for Toxic Substances and Disease Registry provided funding for the project.

The study was designed to estimate the prevalence of elevated serum PCB levels in residents living within a half-mile radius of the

Housatonic River. Participants were administered a questionnaire regarding their residency and occupational histories, fish consumption and recreational activities, especially gardening and activities centered around the river. Questionnaires were ranked with respect to exposure potential, and 120 residents with the greatest opportunity for PCB exposure were invited to provide blood samples for analysis. Sixty-nine individuals provided fasting blood specimens for serum PCB analysis.

Blinded samples were analyzed using methodology developed by the United States Centers for Disease Control and Prevention. Care was taken during sample collection, processing and analysis to avoid any exogenous contamination, which could interfere with proper quantitation of PCBs in the sample. All glassware was solvent rinsed and heated to 130° C prior to use.

Determination of PCB body burden was particularly challenging due to the complexity of the sample matrix, and the sensitivity required measuring part-per-billion concentrations of Aroclor mixtures. Proteins present in the serum were denatured with methanol and removed. PCBs were isolated from the serum by repeated solvent extraction with a mixture of ethyl ether and hexane. Extraneous compounds present in the extracts were selectively removed by silica gel adsorption column chromatography. The "cleaned up" extracts were concentrated in a water bath using a

gentle stream of nitrogen. Concentrated extracts were analyzed by gas chromatography with electron capture detection. Electron capture detectors are extremely sensitive to electronegative compounds such as PCBs. PCBs were separated and identified using a

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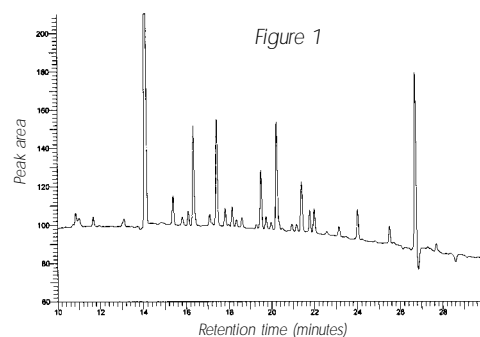


Figure 1. Chromatogram of a study participant with 5.5 ppb Aroclor 1260.

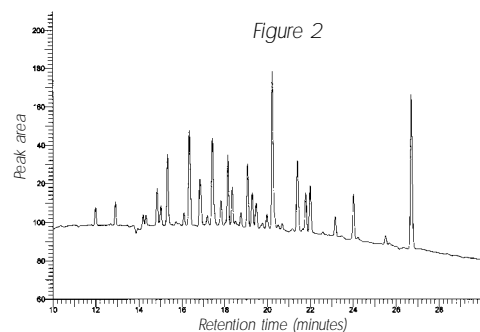


Figure 2. Chromatogram of Aroclor 1260 standard.

Program Reports —

1998 Eastern Equine Encephalitis Surveillance Program Report

by Mary Tobin

From May through early October, or the first frost, eastern equine encephalitis (EEE) surveillance program field workers trap mosquitoes throughout the EEE High Risk area¹. Mosquito collections are brought back to the lab where they are sorted by species and the females are tested for EEE virus (EEEV). In 1998, trapping began on May 5 and was extended until October 17. Seventeen regular sites in eastern Massachusetts were trapped throughout the season using miniature CDC light traps. In addition CO₂ baited traps were set in areas as virus was identified to augment collections of human biting mosquitoes. A total of 44,312 mosquitoes were analyzed, almost twice as many as in 1997. Mosquitoes were tested by plaque assay on chick embryo tissue culture (CETC) followed by immunologic identification by indirect fluorescent assay (IFA). Seven EEEV isolates were identified, all in *Culiseta* (*Cs.*) *melanura*, a species that feeds primarily on birds. The first EEEV isolate of 1998 was identified in mosquitoes trapped on September 21 in Lakeville. EEEV was also isolated from mosquitoes in Kingston, Carver, Easton, Halifax and New Bedford. Highlands J (HJ) virus, an arbovirus not known to cause human disease, but found

in the same environment and often a precursor to EEEV, was also isolated from 45 mosquito pools, including 43 pools of *Cs. melanura*, one pool of *Aedes vexans*, and one pool of *Coquillettida perturbans*.

In addition to collections from Massachusetts' sites, the Rhode Island Department of Environmental Management submitted 701 pools of mosquitoes for the period from June 22 through October 26 for virus analysis. A total of 14 EEEV isolates and 40 HJ virus isolates were identified. Mosquitoes trapped in South Kingston, Westerly, Richmond, and Charlestown, Rhode Island tested positive for EEEV. A total of 251 pools of mosquitoes were also tested from traps set on Hanscom Air Force Base in Middlesex County, MA, from sites on Cape Cod, and from sites in New Hampshire. These mosquitoes were negative for virus.

SLI tests humans, horses and other animals with suspect EEEV infection. In 1998, samples from 24 humans, 6 exotic birds (4 emus, 1 rhea and 1 flamingo), 4 horses, and 1 donkey were submitted for testing. Human serum and cerebral spinal fluid (CSF) samples were examined for antibody

to EEEV by IgM capture enzyme immunoassay (EIA), and indirect fluorescent antibody (IFA) or plaque reduction neutralization assays on CETC. CSF and tissue samples were tested for the presence of virus by plaque assay. Specimens from 4 emus tested positive for EEEV by plaque assay. No other samples had evidence of EEEV infection.

The EEEV positive emus were from herds in Essex and Norton, Massachusetts. In Essex, 18 emus in a herd of 24 animals died between September 27 and October 8. The animals exhibited disease clinically consistent with EEE infection in emus. Unlike infection in humans and horses, which causes neurological symptoms, the infection in adult emus causes a hemorrhagic illness. In the Norton outbreak, 11 juvenile emus from one farm died between October 23 and October 25. This farm is located close to the town of Easton, where EEEV had been identified in *Cs. melanura* collected late in September.

¹ Plymouth, Norfolk, Bristol, Suffolk and eastern Middlesex Counties

Polychlorinated Biphenyl Exposure Assessments

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30 meter, DB-5 (5% phenyl methyl polysiloxane) megabore or open tubular column.

Study samples were quantified as Aroclor 1260, since the chromatographic pattern of the samples most closely resembled this

commercial mixture. Twenty-one peaks were used for identification and quantification purposes (see Figures 1 and 2 on page 2). Aroclor 1260 concentrations ranged from < 2 to 36 parts per billion (ppb) with a mean of 5.4 ppb and a median of 3.9 ppb. As expected, participant age (>65), occupational history and freshwater consumption from the Housatonic River were associated with higher than average PCB levels.

As a follow-up to the Housatonic River Area Exposure Assessment Study, the Environmental Chemistry Laboratory at the SLI continues to offer serum PCB testing as part of the Massachusetts Department of Public Health's on-going risk evaluation and community education program for residents of the eight-town region.

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for the electronic version of this newsletter and other information about the State Laboratory Institute: www.state.ma.us/dph/bls.

Laboratory Training Activities

Public Health Teleconference Series, State Laboratory Institute, Boston, MA: Hepatitis C, May 25; Vancomycin Resistance, June 22; Influenza, October 19; *Chlamydia trachomatis*, November 16. Fee \$25 per site per program. Call (617) 983-6285.

Moving Forward in Cervical Cytology - May 21, Highlander Inn, Manchester, NH: An update for cytologists on new developments in the diagnosis and treatment of cervical cancer. Call (617) 983-6285.

Wet Mounts - June 30 (AM), State Laboratory Institute, Boston, MA: an STD/HIV Prevention Training Center of New England course. Call (617) 983-6945.

State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail: garry.greer@state.ma.us.*

For a list of NLTN courses in your area sign on to the Web at <http://www.cdc.gov/phppo/dls/nlttn.htm>.

The State Laboratory Institute Newsletter is a free monthly publication of the Bureau of Laboratory Sciences

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